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Assessment an antibacterial activity of crud bodies, *Ailolopus thaiossinus* (Orthoptera) and *Polistes watti* larvae (Hymenoptera) by extracted cold and boiled solvents

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Abstract
Todays, insects as adults and immaturs are in focusing of researching new pharmaceutical compounds. Especially, those under habit and habitat stressors. However, insect body extracts have antibacterial activity to current antibiotic resistant bacteria. After the extraction by sequential solvent method, bacterial growth inhibition of dry bodies the insects: grass hopper, *Ailolopus thaiossinus*, larvae of paper wasp, *Polistes watti* and standard antibiotic drug Ceftriaxone (CRO) as positive control against the following bacteria species: Gram - positive *Staphylococcus aureus* and Gram - negatives; *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have been tested. Antibacterial parameter was tested by disc diffusion test. Only *E. coli* and *K. pneumoniae* were sensitive to hexane extract with *A. thaiossinus* and *P. watti* larvae respectively. The two extracts with diethyl ether were exhibited moderate growth inhibition of the tested bacteria except for *P. aeruginosa* resistant to larvae *P. watti* extract. Extracts with ethyl acetate were varied between moderate to resistant activity, Later, methanol extract of the tested insect materals were more effective than the standard drug (CRO) for *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The boiled methanol extract of *A. thaiossinus* more effective than ones of larvae *P. watti* and cold extract of *A. thaiossinus*. In alternative antibiotics, this study as bricks in this field, through practical application the antibacterial compounds as templates for future antibiotic generation.

Keywords: Antibacterial, insect boiled extract, *Ailolopus thaiossinus*, *Polistes watti*

Introduction
Growing of multiple drug resistant bacteria in the last decades were stimulated the searching for new alternatives from different resources with antibacterial properties [1, 2]. Therefore, many researchers discovered new antibacterial ingredients from a wide range of organisms [3, 4, 5]. The various application of insect extracts in folk medicine were directed the scientific attention for developing new antibiotics, which used in treatment the present antibiotic-resistant bacteria [6]. Insects have continues resistance to bacterial infection [7]. Recently, insect products have potential use in medicine, various antimicrobial compounds have been produced by immune system of the insects, which internally biosynthesized as antimicrobial peptides (AMPs), most AMPs are positively charged and consist of 10 – 100 amino acid residues with dominant linear α helical structure [8, 9, 10]. AMPs with low molecular weight from insects as termites, black soldier fly, beetles, caterpillars, and crickets have antimicrobial properties [11]. Also, integument surface products exhibited antimicrobial properties [12]. Some of the identified cuticular lipids of cyclorrhaph flies had antibacterial activity with more effective to Gram positive bacteria [13]. Body extract of black soldier fly inhibited growth of human pathogenic bacteria [14]. The prepared chitosan from grasshopper, *Calliptamus barbarus* chitin have antimicrobial activity against human pathogenic microorganisms with IC50 about 10 mg/ml [15]. Also, extract of the eusocial insects *Apis mellifera*, *Monomorium pharoanis*, *Cremotogastor auberti and camponotus xerxes* had significant inhibition of four pathogenic bacteria in comparison with standard drugs, Ceftriaxone, Gentamycin and Tetracycline antibiotics [16].
The social wasps are feeding with two way adult – larvae trophallaxis habit, the larval saliva contain antibiotic substances provide protection adults and brood in the social wasps, also, vespid larvae are in stress of pathogens that developed in stored food remnants in the peritrophic membrane [17].

On the other hand, venom gland of the social wasps; *Polistes flavus* and *Vespa Orientals* contains peptides with an antibacterial activity against sensitive and resistance *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* [18, 19]. In Iraq, 10 species of vespid family were recorded, two of the genus *Polistes*, *P. gallicus* (Linaeus) and *P. watti* Cameron [20].

In addition to inspecting for new pharmaceutical resources, to rescue developing antibiotic resistance of human pathogenic bacteria. Therefore, the objective of this research is evaluating the antibacterial activity of phytophagous grasshoppers and entomophagous wasp paper larvae insect body extracts as promise medical application in the future.

Materials and Methods

1. Insects

The insects were gathered from their natural habits in Mosul city/ Iraq (34 ° 23′28 N 40 ° 59′16 ″ E): trophalaxed larvae of the social wasp paper, *Polistes watti* and adults of the grass hopper, *Ailolopus thaiossinus*.

2. Bacteria isolates

The isolates of human pathogenic Gram positive bacteria *Staphylococcus aureus*, and Gram negatives *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were selected for their growth inhibition by insect extracts prepared by sequent polar solvents. The stock isolates were presented to the Entomology laboratory as a loan by Dr. alaa Taha Younis / microbiology Lab. – Research Unit of Postgraduates – Biology Department, Collage of Education for Pure Science - Mosul university - Iraq.

3. Agar diffusion test

For determine the antibacterial activity of the insect extract, besides positive control, agar disc diffusion method had been applied after Kirby - Bauer method with minor modification [21, 22].

Prepared extracts by solvent elution

The reared insects were presented to the laboratory and killed by killing jar, Each insect material was dried, powdered by electrical mill and stored at 4 ºC till experimentation. For fraction each crude insect species, the crude was extracted by serial - four stage solvent extraction method after [23] with modifications, the applied solvents were used; hexane, diethyl ether, ethyl acetate and methanol, the obtained soluble constituents by each solvent had been dried and tested their antibacterial activity.

Antibacterial bioassay

For test the antibacterial potency of the sequential fractionated extracts, each bacterium was refreshed from a refrigerated stock. Then, the bacterium samples were subcultured into plate medium. The antibacterial effectiveness of the insect extracts were *in vitro* evaluated by fixing sterilized filter paper discs (Whatmann no1. with 6 mm diameter) in the growth bacteria plates, the discs were previously dipped in 50 mg/ml of each extract for 30 min. and later left for 10 min. for solvent vaporization. 10 mgm ceftriaxone discs were used as positive control. All plates with the implanted discs were incubated at 37 ºC ± 1 for 24 hrs., to allow bacterial growing and clearing by extracted antibacterial compounds. Diameter of the clear Inhibition zone for the tested bacterium was represented by three replications.

The data were tabulated by means and standard deviations. The mean differences were analyzed by ANOVA, Duncan's Multiple Range Test at $p<0.01$ [25].

Results

Extracts efficiency

In order to understand the antibacterial potentials of *A. thalassinus* and *P. watti* larvae extracts, we tested their inhibitory effect on human pathogenic bacteria. Table 1 and 2 shows the antibacterial activity of extracts *A. thalassinus* and *P. watti* larvae, which prepared with gradual sequential polar solvents, the bacterial growth inhibition zone was affected by the applied solvents and bacterial species. Table 1 evokes only *E. coli* inhibited by hexane and ethyl acetate extracts with diameters 7.3 mm, while growth inhibition zones of all the tested bacteria were about 7.5 mm with the diethyl ether. On the other hand, methanol extract more effective on Gram positive than Gram negatives; growth inhibition of *Staph. aureus* 14.7 mm and Gram negatives *E. coli*, *P. auroginosa* and *K. pneumoniae* were 8.3, 9.0 and 11.3 mm respectively. The Standard drug ceftriaxone only inhibited growth *Staph. aureus* and *E. coli* with 13.0 and 40.0 mm, but *P. auroginosa* and *K. pneumoniae* are resistance to this standard drug. Table 2 shows bacteria growth inhibition by *P. watti* larvae extract, hexane extract only inhibited growth *K. pneumoniae* with 9.3 mm. *P. auroginosa* resistance to diethyl ether extract, and inhibition ranged between 7.3 and 8.3 for *E. coli*, *K. pneumoniae* and *Staph. aureus*. Methanol extract was inhibited both *Staph. aureus* and *P. auroginosa* with 14.7 mm, *E. coli* 20.3 mm and the clear zone 11.7 mm for *K. Pneumoniae*.

Table 1: Antibacterial activity of 250 mg/ml of *Ailolopus thaiossinus* extracted by different solvents

Solvent	Inhibition growth zone (mm) of			
	Gram-positive	Gram-negatives		
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***
Hexane	0.0 ± 0.0 b	7.3 ± 0.6 a	0.0 ± 0.0 b	0.0 ± 0.0 b
Diethyl ether	± 0.6 a*7.7*	± 0.6 a*7.7*	± 0.6 a*7.7*	± 0.6 a*7.3*
Ethyl acetate	0.0 ± 0.6 b	7.3 ± 0.6 a	0.0 ± 0.6 b	0.0 ± 0.6 b
Methanol	14.3 ± 1.1 a	8.3 ± 0.6 c	9.0 ± 1.0 c	11.3 ± 0.6 b
+ Ve CRO	13.0 ± 0.6 b	40.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c

- Means with horizontal different letters are significantly different at P ≤ 0.01 (Duncan's test)

Table 2: Growth inhibition of marker bacteria by 250 mg/ml of *Polistes wattii* larvae extract

Solvent	Inhibition growth zone (mm) of			
	Gram-positive	Gram-negatives		
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Hexane	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	9.3 ± 0.6 a
Diethyl ether	± 0.6 a *7.3*	± 0.6 a *7.3*	± 0.0 b *0.0*	± 0.6 a *7.3*
Ethyl acetate	8.3 ± 0.6 a	7.3 ± 0.6 b	0.0 ± 0.0 c	7.7 ± 0.6 ba
Methanol	14.7 ± 0.6 b	20.3 ± 0.6 a	14.7 ± 1.0 b	11.7 ± 0.6 c
+ Ve CRO	13.0 ± 0.6 b	40.0 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c

- Horizontal means ± SDs with different letters are significant different at P≤ 0.01 (Duncan's test)

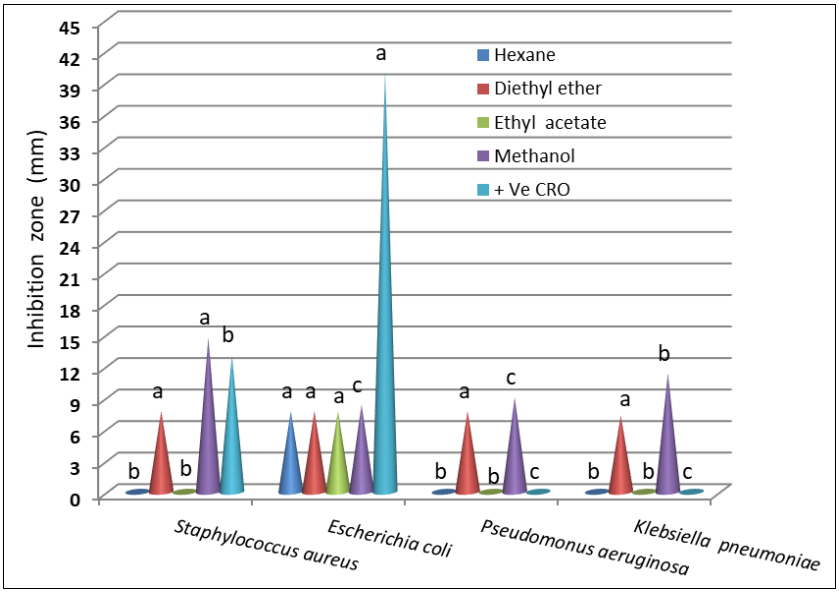

Fig 1: Sensitivity of pathogenic bacteria to *Ailolopus thaiossinus* extracts by sequential solvent polarity method

Inhibition enhancement by sequential extraction

According to the solvents polarity, four stocks of each *A. thalassinus* and larvae of *P. watti* were resulted as four elution times with the following four solvents; hexane, diethyl ether, ethyl acetate and methanol. Growth inhibition zones of the tested bacteria were depended on the solvent had been accomplished. After looking on fig. 1, for *A. thalassinus* stocks; *Staph. aureus*, *P. auroginosa* and *K. pneumoniae* were resistant to hexane and ethyl acetate extracts, but inhibited with clear zones about 7.7 mm for all the tested bacterial species with diethyl ether extract, all the marked bacteria were resistant to ethyl acetate extract except *E coli* inhibited with 7.3 mm, clear zones of *Staph. aureus*, *E. coli*, *P. auroginosa* and *K. pneumoniae* were 14.3, 8.3, 9.0, 11.3 mm respectively. Fig 2 illustrates the diverse resistance and sensitivity with *P. wattii* larvae extract; for *Staph. aureus* was resistant to hexane extract, and inhibited with 7.3, 8.3 and 14.3 mm after treating by diethyl ether, ethyl acetate and methanol extracts respectively. clear zones of *E coli* were ranges between 7.3 and 8.3 mm for the applied four extracts. *P. aurogenosa* resistant to all the extracts except for methanol with 14.3 mm. *K. pneumoniae* inhibition was 7.3 and 7.7 mm for diethyl ether and ethyl acetate, and for hexane and methanol extracts 9.3 and 11.7 mm respectively.

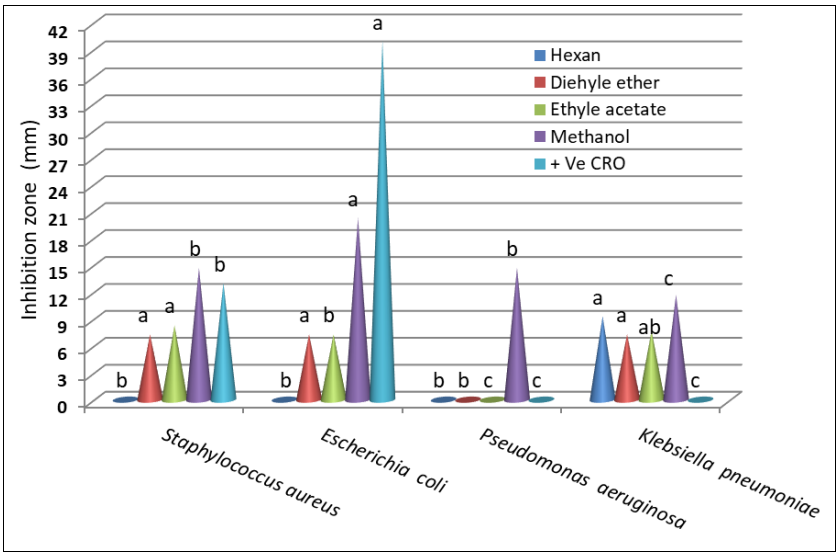

Fig 2: Sensitivity of pathogenic bacteria to *Polistes wattii* larvae extracts by sequential solvent polarity method

Antibacterial activity of boiled and cold extracts

There are variation between methanol hot and cold infusion extracts depending on the insect and bacterium species (table 3). For *A. thalassinus* extract; fig 3 exhibited that hot extract was more inhibited growth *Staph. aureus*, *E coli*, *P. auroginosa* and *K. pneumoniae* at hot (28.3, 24.3, 26.7 and 22.0 mm) than cold (14.7, 8.3, 9.0 and 11.3) extracts. On the other hand, vice versa result with *P. wattii* larvae extract; bacteria treatment with hot extract was gave 7.3 mm inhibition to both *Staph. aureus* and *E coli* and resistant at *P. auroginosa* and *K. pneumoniae*, but for cold extract, the clear zones 14.7, 20.3, 14.7 and 11.7 mm respectively.

Table 3: Growth inhibition of pathogenic bacteria by boiled and cold methanol extracts of *Ailolopus thaiossinus* and larval *Polistes wattii* after sequent solvent refination

Methanol status		Inhibition zone (mm) by			
		Gram- positive	Gram - negatives		
		Staph. aureus	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
A. thaiossinus	Boiled	28.3± 0.6 a	24.3 ± 0.6 c	26.7 ± 0.6 b	22.0 ± 1.0 d
	Cold	14.7 ± 1.1 a	8.3 ± 0.6 c	9.0 ± 1.0 c	11.3 ± 0.6 b
P. wattii	Boiled	7.3 ± 0.6 a	7.3 ± 0.6 a	0.0 ± 0.0 b	0.0 ± 0.0 b
	Cold	14.7 ± 0.6 b	20.3 ± 0.6 a	14.7 ± 1.0 b	11.7 ± 0.6 c

- Means with horizontal different letters are significantly different at $p \leq 0.01$ (Duncan's test)

Discussion

The insects have only innate immunity system and can be easily adapted with habitat contamination by microbial pathogens, through continuous building of antimicrobial active metabolites [14, 7, 25, 26, 11, 14]. The applied insect extracts in the present study had exhibited antibacterial activity against the tested marker bacteria. The growth inhibition zones of any treated bacteria are generally depended on the insect species, solvent polarity that used in the serial extraction and either the last solvent – methanol - used in the sequent extraction boiled nor cold the filtrated stocks. According to Mohtar [27] classification of antibacterial activity of the inset extract, most of the present applied extracts had moderate activity. But it was found meaningful values in comparison with the ceftriaxone antibiotic. For *A. thalassinus*, effect of methanol extract on *Staph. aureus* closes to ceftriaxone, but the bacteria *P. auroginosa* and *K. pneumoniae* were resistant to ceftriaxone, but grass hopper methanol extract had good effect as ranked by Mohtar [27]. The antibacterial effect of *P. wattiii* larvae nearly similar to that of *A. thalassinus* against *Staph. aureus* and *K. pneumoniae*, but more folded against *E coli* and meaningful activity to *P. auroginosa*.

Experimentally, in the present study had been firstly obtained positive results by boiled methanol extracts of the body insects or insect materials with satisfied antibacterial activity in relation to known standard drugs and former studies used cold extracts [28, 29, 30, 14]. In comparison between clear zones of the treated bacteria by boiled and cold extracts (fig 3); high significantly different for boiled *A. thalassinus* than *P. wattii* methanol extracts, and on the other side, cold wasp larvae extract was appeared significant inhibition than that of the grass hopper extract (fig. 3), may due to antibacterial fatty acids and phenolic compounds of the *A. thalassinus* integument had not affected by heating the extract, but for *P. wattii* larvae, antibacterial effect was depended on heat sensitive peptides. In other studies [13, 15, 31], integument constituents had antibacterial activity.

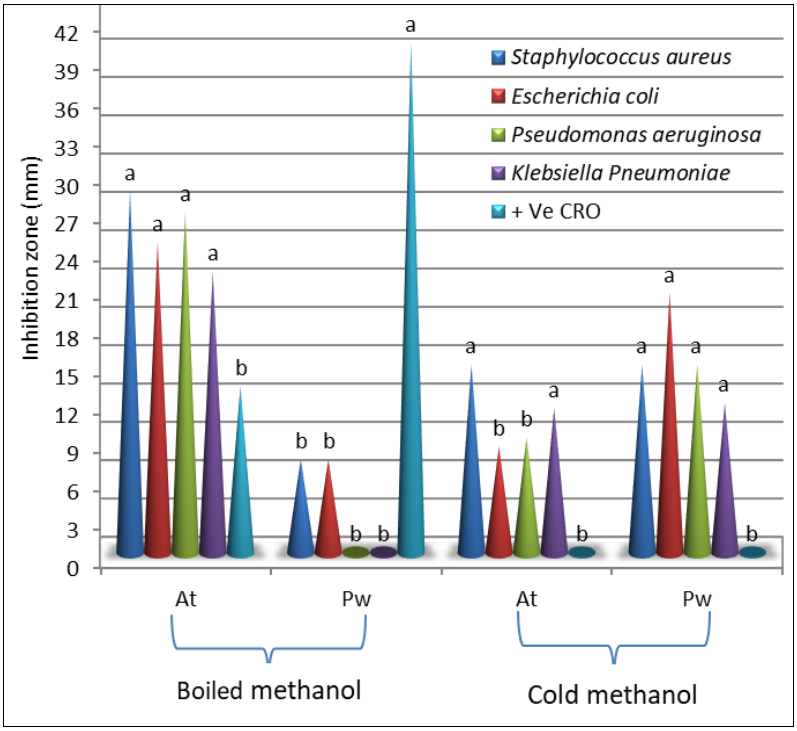

Fig 3: Antibacterial activity of *Ailolopus thaiossinus* (At) and larval *Polistes wattii* (Pw) body extracts, methanol extract was prepared after successful solvent extraction

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